

BOTANICAL GAZETTE

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Artificial cultures of an entomogenous fungus.

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WITH PLATES XIV—XVI.

While making collections in a ravine near Ithaca, N. Y., known as Coy glen, on October 28th, 1893, a specimen of *Isaria farinosa* (Dicks.) Fr. was found upon an arctid chrysalis nestling in the leaf mold, the sporophores of the fungus just projecting above the loose leaves. This plant usually consists of several sporophores, 1–2^{mm} in diameter and 2–4^{cm} in height, issuing from the host. The slender portion of the sporophores, constituting two-thirds to three-fourths of their length, is from whitish to pale yellow or orange yellow in color; while the clavate portion, upon which the fructification is borne, is 2–4^{mm} in diameter, white in color and farinaceous in texture upon the surface. A section of the clavula shows that the interior consists of a mass of hyphæ in arrangement and color similar to those of the sterile portion of the sporophore, being composed of very slender threads lying close together in irregular parallel series. Toward the outer portion of the clavula the threads which arise as branches from the central bundle are whitish and very loosely arranged, forming a fluffy mass. The threads of this fluffy portion branch profusely in a monopodial fashion at first. The terminal branches, very short usually, arise either singly, or opposite, or in whorls. Upon the ends of these final branches the lanceolate basidia are borne in pairs or groups of a varying number. The basidia are surmounted by slender sterigmata which produce the spores in chains much in the same fashion as they are borne in species of *Penicillium*. Indeed the spore clusters when separated resemble very closely the fructification of some species of this genus. The spores are short elliptical or usually rounded, and measure about 2 μ .

Upon reaching the laboratory with the material an attempt was made to cultivate the fungus in ordinary culture media. Accordingly dilution cultures were started in the usual way for the separation of the organism in agar-agar peptone broth, the three dilutions made in culture tubes being poured into Petrie dishes. The cultures were started at about 5 P. M. on the same day as the collection was made, Oct. 28th. On the following morning an examination was made at 10 A. M. No spores were seen which had germinated, though a very thorough search was not made. Oct. 30th, at 9:30 A. M. a second examination was made. Numerous spores had germinated and growth was progressing finely. One or two germ tubes issue from a single spore, and their points of origin, when there is more than one, may be on opposite sides of the spore or on the same side. The general course of the threads at first, when branching does not occur, is quite straight, but the outline of the thread is variously sinuous. Septa probably occur at this stage but they could not be observed while examining the culture in the agar. The protoplasm is very finely granular, and appears to be massed together in certain parts of the thread and spore, the other spaces being occupied with a homogeneous or watery substance. The study of the stages of germination was made from culture no. 1 by placing the Petrie dish upon the stage of the microscope. The spores on the sporophores of the fungus were so numerous and the material was in such a fresh condition that very few foreign organisms appeared in dilutions 1 and 2, while dilution no. 3 was pure, and the separation was effected without any difficulty. From this separation pure cultures were started by transplanting the fungus to culture tubes of ordinary agar, bean stems, and potato. In fact pure cultures were also obtained by touching a flamed platinum needle to the spores on the clavula of the sporophore and then thrusting it into nutrient agar. But the separation was considered necessary in order to have proof in the case of such small germs that the growth obtained was that of the desired plant by watching the germination of the spores and the development of the colonies from these isolated centers in the dilutions.

The fungus grows quite rapidly on artificial media in the culture tubes, soon forming on the surface of the medium a dense velvety growth with quite a long pile. On oblique

slices of potato the larger part of which is exposed, partly dry and not in close contact with the sides of the tube, the fungus spreads quickly, and extends more slowly through the substance of the potato to the surface which is in closer contact with the culture tube, and which is quite moist from the excess of water on the bottom and the side of the tube to which it gravitates as the tube is kept in an oblique position. As the watery infusion gradually disappears by slow evaporation and by being absorbed by the growth of the fungus, the threads appear on the other side of the potato. Now since there is a less content of water and the substance has lost some of its richness, the fungus does not grow so profusely nor so rapidly. There is then a tendency to grow into sporophores composed of numerous parallel threads which arise from the surface of the substratum in the same manner as the normal sporophores of the *Isaria* stage on the natural host, the pupa of the insect. The large majority of these sporophores on potato are much shorter than those on the insect, but they are also much stouter, the diameter being two to four times that of the sterile portion of the sporophore as it appears in nature. From ten to forty of these sporophores may arise from an ordinary sized piece of potato in a culture tube and they are of an orange buff, or buff yellow color. Many of these are from 2-4^{mm} high, while still others are 6-10^{mm}, and they may be divided at the free extremity into several portions. In one culture a very large sporophore was developed which became at length fully 3^{cm} long. It arose perpendicular to the surface of the potato and thus nearly perpendicular to the side of the tube so that when it was 6^{mm} long it came squarely against the wall of the tube. Here the end remained fixed and it appeared for a time as though the sporophore would not grow any longer. From the outer surface of the end which was in close contact with the wall numerous fine radiating threads of the fungus grew out over the inner side of the tube for quite a distance. At the base of this growth the surface presented the farinaceous appearance characteristic of the fructification. In the course of a week it was observed that the sporophore had continued to grow in length and was turned to one side so that its course was downward in the tube. This continued until the entire length of the sporophore was 3^{cm}. At various places it appeared to halt and send out a thin membranous expanded

growth, closely attached to the side of the tube, showing the color of the sterile portion next the wall of the tube, and, on the opposite side, possessing the farinaceous appearance of the fruit. Eventually from several of these expanded portions of the sporophore elongated, radiating, branched, terete fruiting portions were developed, which altogether formed quite a complicated condition of this phase of the plant.

Probably the reason that so many of the sporophores on the potato were very short was due to the fact that the moisture almost entirely disappeared before they were perfected. In every case, however, the free ends of these sporophores were covered with the characteristic fructification.

While no characteristic sporophores are developed at first when there is a large water content and the profuse growth of the fungus forms a long pile covering the substratum, yet spores are developed in great numbers. From these spores on a potato culture pure dilution cultures were started in nutrient agar, in order to study carefully the characteristics of growth and the appearance of the colonies in the artificial medium, as well as the peculiarities of the fructification formed when the sporophores are absent. Dilution cultures were made in order to have the colonies properly separated in the plate. Three dilutions were effected December 28th, at 5:30 P. M., and were poured in Petrie dishes. From no. 1 the study of germination and the development of the colonies was made. December 29th, at 12:30 P. M., the culture was examined. Only a few of the spores were germinating at this time. Those which were immersed in the agar were hyaline in appearance. A few spores here and there were not wholly immersed in the medium, probably owing to the fact that they were dry when the dilutions were made and did not absorb sufficient moisture to permit all of them to sink readily in the liquid. These spores appeared quite dark, as if the wall was dark in color, which resulted from the strong refraction of the light. When these superficial spores germinated, the germ tube penetrated the medium and was hyaline in appearance. Prior to germination the spores swell considerably so that the diameter of the spore is nearly twice what it is when the spores are matured or before they are placed under conditions favorable to germination. Those measured showed a diameter of $3-3.5\mu$. The germ tubes were little more than 2μ in diameter. Many

On December 30th the culture was examined again.

spores had by this time germinated, one to two or three tubes having arisen from a single spore. Branching also by this time was taking place quite freely. Rather faint vacuoles appear in the thread at quite regular intervals as if in the middle of the cell, the transverse walls of which are hard to distinguish in the agar. By December 31st the growth had increased sensibly and the branching was becoming quite profuse while some of the shorter branches were being elevated in the air, but there was as yet no evidence of spore formation.

When the colonies become perceptible to the unaided eye the surface ones are circular, quite compact, and with very fine numerous radiating lines on the margin, giving it a finely fimbriated appearance. When young the deep seated colonies are apt to be angular so that many of them are triangular in form. As the colonies age the superficial ones, or those which reach the surface by later growth, become convex by the elevation of numerous threads which give it a whitish fluffy appearance at the center, while at the margins it is still finely fimbriate from the radiating threads. While the colonies are quite young they resemble those of a species of *Penicillium*, probably *P. glaucum*, which appeared accidentally in culture no. 3. In plate XIV, figs. 2, 3, 4, this single colony of *Penicillium* can be easily differentiated from the colonies of *Isaria*, but in fig. 1 it is impossible to do so except by selecting the corresponding location of the colony in the plate, all the four photographs of the cultures being from the same dish at successive stages of growth. When the *Penicillium* colony fruits the sporophores are quite long and erect and are so arranged that open spaces appear here and there through which the light passes more easily than at other places and a strong differentiation between light and shade appears over different parts of the fruiting portion of the colony. There is also very little of the fluffy arrangement of the aerial hyphæ, such as occurs in the *Isaria* colonies.

As the colonies of *Isaria* become more and more elevated from the medium they become mealy white in appearance from the numbers of spores produced, mixed with the mass of cottony threads. The appearance of the colonies may be varied somewhat by periodic growth, induced by variations in the temperature. Some tests of this were made with the culture no. 3 of the dilution for the separation of the fungus.

After growing for some time in a rather cool room, at a temperature ranging from $15-18^{\circ}\text{C}.$, the culture was placed in the thermostat with a temperature of $25.6^{\circ}\text{C}.$ In a few days a profuse growth had taken place, making a distinct concentric ring. At the center was a strongly convex dense portion, separated from an outer ring which was elevated above the intervening portion. In the thermostat at the higher temperature this ring frequently became elevated considerably above the center of the colony. The margin of the colony presented a larger corona of radiating threads than would have appeared had the culture been kept at the lower temperature for the same time.

By January 12th the colonies from the pure culture started Dec. 28th, examined with a low power of the microscope, show the loose cottony mass to be composed of numerous interwoven threads bearing short sporophores consisting of a single thread. Usually these were arranged in a monopodial fashion but sometimes they were opposite. These correspond to the ultimate branches of the external layer of the clavula on the natural sporophores. Like them they are surmounted by several short lanceolate basidia, the sterigmata of which bear long chains of spores, reminding one very forcibly of the fructification of a *Penicillium*, though on shorter sporophores.

The illustrations in plate XIV are natural size reproductions of culture no. 3 at different stages of growth. In figs. 1 and 2, the colonies were not yet elevated above the medium, and, being transparent and very delicate, could not be photographed by reflected light to show the peculiar characters. In figs. 3 and 4, the colonies were elevated at the center above the medium. Figure 3 was photographed by transmitted light to show the finely fimbriated margin of the colonies and the relation of the same to the denser portion of the colony. This photograph is not as good as it should be under favorable circumstances, since by this time the medium had become milky in color from the entrance of some species of bacteria which had accidentally gotten into the culture, a small colony of which can be seen on the upper right margin. The light transmitted through the milky portion of the medium also affected the sensitive plate and the differentiation between the colonies and the intervening spaces was not strong. It is sufficiently so, however, to show

the character of the margin of the colonies. By transmitted light the elevation of the colonies could not be shown. This can be done by photographing in the ordinary way by reflected light. Such a photograph taken on the same day is reproduced in figure 4, of the same plate. The margin of the colonies, however, is not shown by this process, but a knowledge of the true character of the colonies can be obtained by putting the two photographs together.

Several cultures on artificial media in culture tubes have been made but in no case has any thing resulted which shows the perfect or ascigerous stage of the fungus. Upon nutrient agar, nutrient gelatine, and bean stems, nothing but the cottony or fluffy growth, covered by the farinaceous fructification, appears. On potato this growth first appears, to be succeeded by the characteristic fructification of the *Isaria* stage.

Tulasne has shown¹, not by cultural experiments, but by contiguity of development, that *Isaria farinosa* (Dicks.) Fr. is the conidial stage of *Cordyceps militaris* (Linn.) Link. A large number of cultures, perhaps varying the substratum and other conditions of environment, might result in the development of the *Cordyceps* form in artificial cultures from the *Isaria* stage.

The fact that the *Isaria* stage will develop readily on various media such as described above is evidence that it can develop readily as a saprophyte, and is thus more likely to be preserved in greater abundance and in wider distribution than if it were able to propagate itself only on insects.

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EXPLANATION OF PLATES XIV-XVI.

PLATE XIV.—Fig. 1. Photograph, nat. size, by transmitted light, of plate culture in agar, showing colonies.—Fig. 2, same at more advanced stages of growth.—Fig. 3, same at still more advanced stage showing the fimbriated margin of colonies.—Fig. 4, same by direct light to show elevation of colonies.

PLATE XV.—Fig. 5, germinating spores.—Fig. 6, farther advanced stage.—Fig. 7, group of fruiting basidia from sporophore of plant developed under natural conditions.—Figs. 8 and 9, same, from culture on agar.

PLATE XVI.—Fig. 10. Photograph of *Isaria farinosa* from which cultures were started; magnified twice.—Fig. 11, fructification in elevated portion of colony on agar.

In plates XV and XVI the scale shown is 1 mm magnified about 18 times. Figs. 5, 6, 7, 8, 9, 11 are magnified 50 times more than the scale. Drawn by aid of camera lucida.

¹Note sur les *Isaria* et *Sphæria* entomogenes. Ann. d. Sci. Nat. Bot. IV. 8: 35. 1857.—*Torrubia militaris*. Selecta Fung. Carp. 3: 6. 1865.